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TOXICITY OF "DETOXIFIED" GB, VX, AND HD TO ANIMALS AND AQUATIC ORGANISMS

E. J. Owens, et al

Edgewood Arsenal
Aberdeen Proving Ground, Maryland

June 1973

AD

EDGEWOOD ARSENAL
TECHNICAL REPORT

EATR 4755

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by

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June 1973

DEPARTMENT OF THE ARMY
Headquarters, Edgewood Arsenal
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13. ABSTRACT

A study was conducted to establish the procedures necessary to assure that "detoxified" GB, VX, and HD would not be harmful to personnel handling the solutions and that solutions disposed of into water systems would not be harmful to people and aquatic organisms. The solutions tested were: 10% sodium carbonate, pH 12.3; 10% calcium hypochlorite, pH 12.6; GB detoxified with 10% sodium carbonate, pH 10.5; VX detoxified with 10% calcium hypochlorite, pH 5.9; and HD detoxified with 10% calcium hypochlorite, pH 3.6. Solutions neutralized to pH 7 were also tested. Based on intravenous, intragastric, ocular, and cutaneous tests of the above solutions in mammals, immersion of fish and microcrustacea, and growth of aquatic plants, the following conclusions were reached: (1) Personnel handling the unneutralized solutions should wear the same body protection prescribed for handling corrosive liquids. (2) The solutions should be diluted 1:1,000 to 1:10,000 before being handled by unprotected personnel. (3) Unneutralized 10% calcium hypochlorite should be diluted to <10 ppm and the other solutions should be diluted to <50 ppm before release into waters inhabited by fish. (4) All solutions must be neutralized before being released into waters containing aquatic plants; the GB and VX solutions should also be diluted 1:100,000 and the HD solution should be diluted 1:1,000,000. These proposals must be qualified by the fact that the tests were done for one purpose: to develop toxicity data for use in establishing procedures for intermittent disposal of small lots of detoxified VX, GB, and HD in brackish or salt water streams. The plants and aquatic species tested are indigenous to North America. Also, because it was anticipated that enough time would elapse between "dumps" to avoid buildups in the water, only acute studies were done. Thus, the effects of chronic exposure to the levels proposed are not known. These facts should be taken into consideration in any attempt to apply the data to areas other than brackish or salt water streams in North America or to a long-term disposal of large amounts of the solutions studied.

14. KEYWORDS

Decontamination solutions
Toxicity
VX
GB
HD
Mammalian toxicity
Aquatic toxicity
Plant toxicity
Hazard definition

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June 1973

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Task 1W662710AD6301

DEPARTMENT OF THE ARMY
Headquarters, Edgewood Arsenal
Aberdeen Proving Ground, Maryland 21010

FOREWORD

The work described in this report was authorized under Task 1W662710AD6301, Chemical Safety Investigations, Assessment and Control of Chemical Contaminants. This work was started in May 1971 and completed in April 1972.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

Acknowledgments

The research described was performed jointly by the Aerosol and Basic Toxicology Branches, Toxicology Division, and the Experimental Medicine Branch, Medical Research Division. Although separate, more detailed reports may be published later, the work of each branch has been combined in this document to provide a ready source for those who must make decisions on disposal of the materials studied.

DIGEST

A study was conducted to establish the procedures necessary to assure that "detoxified" BZ, VX, and HD would not be harmful to personnel handling the solutions and that solutions disposed of into water systems would not be harmful to people and aquatic organisms. The solutions tested were:

10% sodium carbonate, pH 12.3
10% calcium hypochlorite, pH 12.6
GB detoxified with 10% sodium carbonate, pH 10.5
VX detoxified with 10% calcium hypochlorite, pH 5.9
HD detoxified with 10% calcium hypochlorite, pH 3.6

Solutions neutralized to pH 7 were also tested.

Based on intravenous, intragastric, ocular, and cutaneous tests of the above solutions in mammals, immersion of fish and microcrustacea, and growth of aquatic plants, the following conclusions were reached:

1. Personnel handling the unneutralized solutions should wear the same body protection prescribed for handling corrosive liquids.

2. The solutions should be diluted 1:1,000 to 1:10,000 before being handled by unprotected personnel.

3. Unneutralized 10% calcium hypochlorite should be diluted to <10 ppm and the other solutions should be diluted to <50 ppm before release into waters inhabited by fish.

4. All solutions must be neutralized before being released into waters containing aquatic plants: the GB and VX solutions should also be diluted 1:100,000 and the HD solution should be diluted 1:1,000,000.

These proposals must be qualified by the fact that the tests were done for one purpose: to develop toxicity data for use in establishing procedures for intermittent disposal of small lots of detoxified VX, GB, and HD in brackish or salt water streams. The plants and aquatic species tested are indigenous to North America. Also, because it was anticipated that enough time would elapse between "dumps" to avoid buildups in the water, only acute studies were done. Thus, the effects of chronic exposure to the levels proposed are not known.

These facts should be taken into consideration in any attempt to apply the data to areas other than brackish or salt water streams in North America or to a long-term disposal of large amounts of the solutions studied.

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TOXICITY OF "DETOXIFIED" GB, VX, AND HD TO ANIMALS AND AQUATIC ORGANISMS

I. INTRODUCTION.

The following Disposition Form, quoted in part below, dated 8 December 1970, subject: Disposal of Waste Solutions Generated from Chemical Reactions of Agents and Decontaminating Solutions, was forwarded to the Director, Research Laboratories, by the Associate Technical Director, Edgewood Arsenal:

> *1. Edgewood Arsenal has been directed to develop procedures and equipment to demilitarize limited quantities of unserviceable GB, VX, and H/HD-filled munitions at various storage locations. Methods have been conceived and associated equipment designed and fabricated by Wpn Dev and Engr Labs in conjunction with established guidelines generated from the current atmosphere of absolute safety. Enactment of PL91-441 requires review of disposal procedures by DHEW prior to commencing operations.*

This is a report of the studies that were performed to provide information required to adequately characterize the toxicity of chemical wastes.

II. EXPERIMENTAL PROCEDURES AND RESULTS.

A. Test Materials.

The following test solutions were freshly prepared by the Chemical Process Laboratory on the day of use by the Biomedical Laboratory:

10% sodium carbonate (Na_2CO_3), pH 12.3
10% calcium hypochlorite $Ca(OCl)_2$, pH 12.6
GB detoxified with 10% sodium carbonate, pH 10.5 (16.4 gm of GB per liter of decontaminant, agitated for 4 hours)
VX detoxified with 10% calcium hypochlorite, pH 5.9 (16.4 gm of VX per liter of decontaminant, agitated for 4 hours)
HD detoxified with 10% calcium hypochlorite, pH 3.6 (12.0 gm of HD per liter of decontaminant, agitated for 24 hours)

Portions of the five solutions were adjusted to pH 7 as follows:

10% sodium carbonate: 1.0 N hydrochloric acid
10% calcium hypochlorite: 1.0 N hydrochloric acid
100 ml of GB:sodium carbonate slurry: 20.4 ml of 5.5 N hydrochloric acid and 10 ml of 0.1 N hydrochloric acid
25 ml of VX:calcium carbonate solution: 0.5 ml of 1.0 N sodium hydroxide and 1.3 ml of 0.1 N hydrochloric acid
25 ml of HD:calcium carbonate solution: 0.7 ml of 1.0 N sodium hydroxide and 2.4 ml of 0.1 N hydrochloric acid

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B. Intravenous, Lymph Sac, Intragastric, Ocular, and Cutaneous Tests.

1. Intravenous and Lymph Sac Toxicity Tests.

The solutions, without further dilution, were injected into the tail veins of the mouse and rat, the ear vein of the rabbit, and the dorsal lymph sac of the frog. The LD50's were based on mortalities occurring during a 30-day observation period. A summary of the LD50's is shown in table I. Additional data are shown in tables A-I and A-II, appendix.

The toxicities of the 10 solutions (ml/kg) were then ranked, with the number one being assigned to the most toxic. The rankings are shown in table II. The 10% calcium hypochlorite solution (pH 12.6) was the most toxic in all species tested; when this solution was neutralized, it was the second most toxic. VX:calcium hypochlorite (pH 5.9 and pH 7.0) and HD:calcium hypochlorite (pH 3.6 and pH 7.0) had an LD50 of less than 1 ml/kg in the rabbit and the rat.

All solutions containing sodium carbonate had LD50's of 1.8 ml/kg or higher in every species.

Neutralization did not lower the toxicity of the decontaminated agent solutions.

Table III is a comparison of the intravenous LD50's of undetoxified and detoxified GB, VX, and HD. Undetoxified VX and GB were from three to five orders of magnitude more toxic than the byproducts in the detoxified solutions. The detoxified HD was almost as potent as the undetoxified agent. However, in the cutaneous studies described later, the product of the HD:calcium hypochlorite reaction did not blister rabbit skin.

Although the LD50's of the undetoxified agents were not established in frogs, results with a few frogs given GB indicate its LD50 would be greater than 1 mg/kg.

2. Intragastric Toxicity Tests.

The intragastric toxicity of each of the 10 solutions was established in the mouse, rat, and rabbit. The animals were not fed for 24 hours before dosing, but water was available to them. The undiluted solutions were delivered from a syringe into an esophageal catheter; a small amount of water was then squirted into the catheter to flush any residue into the stomach. LD50's were based on deaths occurring during a 30-day observation period.

A summary of the results of these tests is shown in table IV, and details are shown in tables A-I and A-II, appendix. The toxicities of the 10 solutions (ml/kg) were ranked, with the number 1 being assigned to the most toxic. The rankings are shown in table V.

Unneutralized calcium hypochlorite and neutralized GB:sodium carbonate were about equal in toxicity and were the most toxic of the 10 materials tested. Neutralized calcium hypochlorite ranked third in the series. No relationship between toxicity and detoxicant was obvious, although in the intravenous studies we had found that those mixtures containing calcium hypochlorite were usually the most toxic.

The range of responses for the three species was much narrower when the materials were given intragastrically than when they were given intravenously; i.e., 13.3-56.9 ml/kg versus 0.18->31.6 ml/kg.

Table I. Intravenous and Lymphatic Toxicities of Detoxicants and Agent-Detoxicant Solutions

Solution	LD50											
	Mouse			Rat			Rabbit			Frog*		
	Solution	Decontaminant	Agent**	Solution	Decontaminant	Agent**	Solution	Decontaminant	Agent**	Solution	Decontaminant	Agent**
	ml/kg	mg/kg	mg/kg	ml/kg	mg/kg	mg/kg	ml/kg	mg/kg	mg/kg	ml/kg	mg/kg	mg/kg
10% Na_2CO_3, pH 12.3	4.7	470	-	3.0	300	-	1.8	178	-	>31.6	>3160	-
10% $Ca(OCl)_2$, pH 12.6	0.86	86	-	0.18	18	-	0.18	18	-	1.78	178	-
GB:10% Na_2CO_3, pH 10.5	7.1	-	117	2.6	-	43	1.8	-	29.2	31.6	-	518
VX:10% $Ca(OCl)_2$, pH 5.9	2.4	-	39	1.22	-	20	0.74	-	12	>31.6	-	>518
HD:10% $Ca(OCl)_2$, pH 3.6	2.0	-	24	0.70	-	8	1.8	-	21.4	31.6	-	379
NEUTRALIZED TO pH 7												
10% Na_2CO_3	13.8	1380	-	3.5	353	-	8.1	814	-	>28.6	>2860	-
10% $Ca(OCl)_2$	2.4	238	-	1.03	103	-	0.51	51	-	>30.0	>3000	-
GB:10% Na_2CO_3	7.8	-	128	2.8	-	46	5.6	-	92.2	>31.6	-	>518
VX:10% $Ca(OCl)_2$	2.4	-	39	1.39	-	23	0.56	-	9.2	>31.6	-	>518
HD:10% $Ca(OCl)_2$	2.17	-	26	0.75	-	9	1.33	-	16	>31.6	-	>379

* Injected in the dorsal lymph sac.

** Originally in the volume of the LD50.

Table II. Ranking of Intravenous and Lymphatic Toxicities of Detoxicants and Detoxified GB, VX, and HD

Solution	Ranking* of LD50's by species				LD50's for solutions (all species)	
	Mouse	Rat	Rabbit	Frog	Range	Rank*
					ml/kg	
10% Na_2CO_3, pH 12.3	5	9	6	5	1.8->31.6	7
10% $Ca(OCl)_2$, pH 12.6	1	1	1	1	0.18-1.78	1
GB:10% Na_2CO_3, pH 10.5	6	7	6	4	1.8-31.6	7
VX:10% $Ca(OCl)_2$, pH 5.9	4	5	4	5	0.74->31.6	5
HD:10% $Ca(OCl)_2$, pH 3.6	2	2	6	4	0.70-31.6	4
NEUTRALIZED TO pH 7						
10% Na_2CO_3	8	10	8	2	3.5->28.6	9
10% $Ca(OCl)_2$	4	4	2	3	0.51->30.0	2
GB:10% Na_2CO_3	7	8	7	5	2.8->31.6	8
VX:10% $Ca(OCl)_2$	4	6	3	5	0.56->31.6	3
HD:10% $Ca(OCl)_2$	3	3	5	5	0.75->31.6	6

* Number 1 is the lowest LD50.

Table III. Comparative Toxicities of Active and Detoxified (Unneutralized) GB, VX, and HD Administered Intravenously

Agent	LD50*		
	Mouse	Rat	Rabbit
		mg/kg	
GB			
Active	0.100-0.140	0.045	0.015
Detoxified with 10% Na_2CO_3	117.0	43.0	29.2
VX			
Active	0.014	0.008	0.0084
Detoxified with 10% $Ca(OCl)_2$	39.0	20.0	12.0
HD			
Active	8.6	3.3	4.0
Detoxified with 10% $Ca(OCl)_2$	24.0	8.0	21.4

* For detoxified solutions, the original amount of agent in the volume of the LD50.

Table IV. Intragastric Toxicities of Detoxicants and Agent-Detoxicant Solutions

Solution	LD50								
	Mouse			Rat			Rabbit		
	Solution	Decontaminant	Agent*	Solution	Decontaminant	Agent*	Solution	Decontaminant	Agent*
	ml/kg	mg/kg	mg/kg	ml/kg	mg/kg	mg/kg	ml/kg	mg/kg	mg/kg
10% Na_2CO_3, pH 12.3	43.7	4370	-	31.9	3190	-	21.5	2150	-
10% $Ca(OCl)_2$, pH 12.6	21.0	2100	-	13.9	1390	-	17.8	1780	-
GB:10% Na_2CO_3, pH 10.5	36.9	-	605	24.3	-	399	30.4	-	499
VX:10% $Ca(OCl)_2$, pH 5.9	48.2	-	790	40.8	-	669	23.7	-	389
HD:10% $Ca(OCl)_2$, pH 3.6	39.8	-	478	29.7	-	356	31.6	-	379
NEUTRALIZED TO pH 7									
10% Na_2CO_3	38.0	3800	-	29.3	2930	-	14.6	1460	-
10% $Ca(OCl)_2$	40.6	4060	-	30.3	3030	-	21.5	2150	-
GB:10% Na_2CO_3	27.7	-	454	27.8	-	456	13.3	-	218
VX:10% $Ca(OCl)_2$	56.9	-	933	41.2	-	676	17.8	-	292
HD:10% $Ca(OCl)_2$	36.0	-	432	31.7	-	380	17.8	-	214

* Originally in the volume of the LD50.

Table V. Ranking of Intragastric Toxicities of Detoxicants and Detoxified GB, VX, and HD

Solution	Ranking* of LD50's by species			LD50's for solutions (all species)	
	Mouse	Rat	Rabbit	Range	Rank*
				ml/kg	
10% Na_2CO_3, pH 12.3	8	8	4	21.5-43.7	5
10% $Ca(OCl)_2$, pH 12.6	1	1	3	13.9-21.0	2
GB:10% Na_2CO_3, pH 10.5	4	2	6	24.3-36.9	7
VX:10% $Ca(OCl)_2$, pH 5.9	9	9	5	23.7-48.2	6
HD:10% $Ca(OCl)_2$, pH 3.6	6	5	7	29.7-39.8	8
NEUTRALIZED TO pH 7					
10% Na_2CO_3	5	4	2	14.6-38.0	3
10% $Ca(OCl)_2$	7	6	4	21.5-40.6	5
GB:10% Na_2CO_3	2	3	1	13.3-27.8	1
VX:10% $Ca(OCl)_2$	10	10	3	17.8-56.9	4
HD:10% $Ca(OCl)_2$	3	7	3	17.8-31.7	4

* Number 1 is the lowest LD50.

Neutralization did not reduce the potency of the decontaminated solutions in all cases.

Table VI is a comparison of the intragastric toxicities of the undetoxified agents and the detoxified agent solutions in the rat and the rabbit (no intragastric LD50's for the active agents were established in mice). The undetoxified agents were between two and three orders of magnitude more toxic than the byproducts in the detoxified solutions.

Table VI. Comparative Toxicities of Active and Detoxified (Unneutralized) GB, VX, and HD Administered Intragastrically

Agent	LD50*	
	Rat	Rabbit
	mg/kg	
GB		
Active	0.870	2.50
Detoxified with 10% Na_2CO_3	399	499
VX		
Active	0.100	0.123
Detoxified with 10% $Ca(OCl)_2$	669	389
HD		
Active	17.0	–
Detoxified with 10% $Ca(OCl)_2$	356	–

* For detoxified solutions, the original amount of agent.

3. Ocular and Cutaneous Tests.

a. Ocular Testing.

Each rabbit selected for ocular testing had been carefully examined, including fluorescein staining of the cornea, to exclude those having eye damage.

Six rabbits per solution (only the neutralized sodium carbonate and calcium hypochlorite were omitted from this test) were used. One-tenth milliliter was instilled in the right eye; the left eye served as a control. The animals were restrained for the first 24 hours to prevent them from pawing their eyes or faces. After this 24-hour period, the eyes were flushed with isotonic saline and wiped with gauze. Clinical observations were recorded. Then one drop of fluorescein sodium ophthalmic solution U.S.P. was instilled into each eye, and the eyes were flushed with saline and observed under ultraviolet light for corneal damage. This procedure was repeated 2, 5, 7, 14, 21, and 28 days after dosing.

Evaluation of eye effects was in accordance with the modified Draize technique.[1] The grades for ocular effects are shown in table VII.

The only solutions that affected the eyes were the unneutralized sodium carbonate and calcium hypochlorite solutions (table VIII).

Rabbits dosed with calcium hypochlorite showed mild chemosis, severe redness, some corneal damage, and mild iritis 24 hours after dosing. These effects became more severe during the next 7 days and then gradually subsided during the second week. After 20 days, all eyes were normal.

Rabbits dosed with sodium carbonate had no immediate effect, but in 24 hours three rabbits displayed redness and two of these also had mild chemosis. Two of the three rabbits recovered after 48 hours; and the other, in less than 5 days.

No signs of systemic toxicity were seen in any rabbit.

b. <u>Cutaneous Testing.</u>

The cutaneous tests were conducted at the same time as the ocular tests and in the same rabbits. The back of each rabbit was clipped and carefully examined; those having skin defects were not used. One-half milliliter of each solution was applied to the center of the bare area, and the trunk of the restrained rabbit was wrapped in a plastic sleeve to occlude the dose. After 24 hours, the sleeves were removed and the rabbits' backs were examined for irritation in accordance with the modified Draize technique.[1,2] The criteria are listed in table IX. The examinations were repeated at 2, 5, 7, 14, 21, and 28 days.

Only the unneutralized calcium hypochlorite had any cutaneous effects (table VIII). Redness appeared at 24 hours and necrosis at 48 hours in all six rabbits. Patches of eschar were seen for 14 days after dosing. After 20 days, all skin areas were normal.

C. <u>Aquatic Vertebrate and Invertebrate Testing.</u>

1. <u>Aquatic Vertebrate Toxicity Tests.</u>

a. <u>Materials and Methods.</u>

The aquatic species tested were white perch *(Morone americana)* weighing an average of 1.2 oz and measuring 5 to 6 inches in length, and striped bass *(Roccus saxatilis)*, weighing an average of 0.5 oz and measuring 2 to 3 inches in length. Both species were seined on the morning of the test days from several locations on Gunpowder Neck along the Bush and Gunpowder Rivers.

Twenty-four hours before each toxicity test, six 25-gallon stainless steel containers were filled with 100 liters of water freshly obtained from White Oak Point on Carroll's Island. The water was brought to laboratory temperature (71° ± 1°F; 21.7°C), and it was aerated for 24 hours before the fish were added.

The five unneutralized solutions used in the mammalian toxicity tests were used in these studies. The solutions were added to the aerated water in the stainless steel containers 30 minutes before the fish were transferred from a holding tank. Uniform distribution of the solutions throughout the water was assured by aeration and stirring. The water in one of the six containers was not contaminated; the animals placed in this water served as normal controls. The range of levels of contamination were as follows:

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1 Illustrated Guide for Grading Eye Irritation by Hazardous Substances. Food and Drug Administration. November 1967.

2 Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics. Association of Food and Drug Officials of the United States, Baltimore, Maryland. 1959.

Table VII. Gradations of Eye Effects

Ocular effect	Grade
Cornea (C)	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	1*
Easily discernible translucent areas, details of iris slightly obscured	2
Nacreous areas, no details of iris visible, size of pupil barely discernible	3
Iris (I)	
Normal	0
Markedly deepened folds, congestion, swelling, moderate circum-corneal injection (any of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)	1*
No reaction to light, hemorrhage, gross destruction (any or all of these)	2
Conjunctival Redness (R) (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
Vessels normal	0
Some vessels definitely injected	1
Diffuse, crimson red, individual vessels not easily discernible	2*
Diffuse beefy red	3
Chemosis (CH)	
No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about half closed	3
Swelling with lids more than half closed	4

* Indicates lowest grades considered positive under Section 191.12 of the Federal Hazardous Substances Labeling Act Regulations.

Table VIII. Ocular (0.1 ml) and Cutaneous (0.5 ml) Effects of Detoxified GB, VX, and HD Solutions in Rabbits (Six per Solution)

Solution	Site of effect	Gradation of effects*						
		1 day	2 days	5 days	7 days	14 days	21 days	28 days
GB:10% Na_2CO_3, pH 10.5	Ocular	0	0	0	0	0	0	0
	Cutaneous	0	0	0	0	0	0	0
GB:10% Na_2CO_3, pH 7	Ocular	0	0	0	0	0	0	0
	Cutaneous	0	0	0	0	0	0	0
10% Na_2CO_3, pH 12.3	Ocular	CHI and RI-2 (2/6)** RI (1/6) O (3/6)	RI (1/6) O (5/6)	0	0	0	0	0
	Cutaneous	0	0	0	0	0	0	0
VX:10% $Ca(OCl)_2$, pH 5.9	Ocular	0	0	0	0	0	0	0
	Cutaneous	0	0	0	0	0	0	0
VX:10% $Ca(OCl)_2$, pH 7.0	Ocular	0	0	0	0	0	0	0
	Cutaneous	0	0	0	0	0	0	0
HD:10% $Ca(OCl)_2$, pH 3.6	Ocular	0	0	0	0	0	0	0
	Cutaneous	0	0	0	0	0	0	0
HD:10% $Ca(OCl)_2$, pH 7	Ocular	0	0	0	0	0	0	0
	Cutaneous	0	0	0	0	0	0	0
10% $Ca(OCl)_2$, pH 12.6	Ocular	CH2 (6/6) R3 (5/6) C2 (5/6) I1 (1/6)	CHI-3 (6/6) RI-2 (6/6) CI-3 (3/6) I1 (1/6)	CHI-3 (5/6) RI-2 (5/6) C3 (1/6)	CHI-3 (4/6) O (1/5)	CHI (1/6) O (5/6)	0	0
	Cutaneous	EI-3 (6/6)	N2-3 (6/6)	N2-3 (6/6)	D3 (1/6) N2 (3/6) O (2/6)	NI-2 (2/6) O (4/6)	0	0

* See tables VII and IX for descriptions of abbreviations and grading system.
** Numbers in parentheses are fractions of animals responding.

Table IX. Gradations of Skin Effects

Skin effect	Grade
Erythema	
No erythema	0
Mild erythema	1
Moderate erythema	2
Severe erythema	3
Erythema with edema	4
Necrosis	
No necrotic tissue	0
Less than 50% necrotic tissue	1
50% to 100% necrotic tissue	2
100% necrotic tissue with well-defined eschar formation	3
Dehydration and Desquamation	
No dehydration or desquamation	0
Mild dehydration or desquamation	1
Moderate dehydration or desquamation	2
Severe dehydration or desquamation	3

White perch:

1,000-10,000 ppm of 10% sodium carbonate
5-1,000 ppm of 10% calcium hypochlorite
1,000-10,000 ppm of GB:sodium carbonate
100-10,000 ppm of VX:calcium hypochlorite
100-10,000 ppm of HD:calcium hypochlorite

Striped bass:

750-4,000 ppm of sodium carbonate
5-80 ppm of calcium hypochlorite
1,000-6,000 ppm of GB:sodium carbonate
50-2,000 ppm of VX:calcium hypochlorite
1,000-6,000 ppm of HD:calcium hypochlorite

Ten fish of each species were used at each concentration. They were observed continuously for 24 hours and the deaths were recorded in minutes from the start of the time of their exposure to the solutions.

The mortality data were analyzed on the basis of the reciprocal of time to response. In this method, the number of animals (percentage of population) dying at each concentration was tabulated for each time period. From the tabulation, a Bliss dose (concentration) versus percentage response regression line was developed for each time interval. From each Bliss line, the points for 1%, 16%, 30%, 50%, 84%, and 99% population responses were extracted. These points for each concentration were then regressed against time over all intervals, using the following equation:

$$\text{Log } D = a + b(1/T)$$

where

a = intercept

b = slope

D = dose (concentration)

T = time

This resulted in a series of curves showing concentration and time that the six percentage levels of the population will respond. This method is preferred over linear regression analysis because extrapolation beyond the limits of experimental evidence is virtually eliminated.

b. Results.

The results are shown in tables X through XIX and in figures 1 through 5. Table XX is a summary of the toxicities in the perch and it compares a ranking of the combined intravenous toxicities of each solution in all mammalian species tested with those in perch. Calcium hypochlorite was the most toxic of the solutions in both fish and mammals. The other solutions had similar rankings in both fish and mammals.

Table X. Toxicity of 10% Sodium Carbonate Solutions in White Perch

Concentration	Lt50	Cumulative mortality*	Time to death
ppm	min		min
1,000		0/10	
2,000		0/10	
2,500	1157	5/10	941, 999, 1218, 1286, 1408
5,000	1180	3/10	989, 1244, 1334,
6,000	779	10/10	468, 555, 675, 799, 821, 840, 861, 911, 926, 1168
10,000	501	10/10	367, 378, 382, 418, 540, 540, 561, 625, 660, 660

* 24-hr Observation period.

Table XI. Toxicity of 10% Calcium Hypochlorite Solutions in White Perch

Concentration	Lt50	Cumulative mortality*	Time to death
ppm	min		min
5		0/10	
7.5		0/10	
10	644	9/10	323, 508, 511, 543, 569, 771, 778, 928, 1317
50	117	10/10	74, 95, 97, 100, 100, 101, 102, 130, 156, 328
100	36	10/10	21, 33, 35, 36, 37, 38, 40, 42, 43, 44
1,000	5	10/10	2, 3, 3, 4, 5, 7, 7, 8, 8, 10

* 24-hr Observation period.

Table XII. Toxicity of GB:Sodium Carbonate Solutions in White Perch

Concentration	Lt50	Cumulative mortality*	Time to death
ppm	min		min
1,000		0/10	
2,000		1/10	1203
2,500	741	9/10	305, 510, 695, 717, 890, 915, 975, 975, 1125
3,000	192	10/10	144, 145, 161, 179, 194, 195, 199, 206, 254, 291
5,000	>241	10/10	195, 224, 226, 242, 268, 357, 373, 386, 463, 559
5,000		10/10	157, 160, 162, 176, 178, 187, 189, 217, 222, 242
10,000	312	10/10	213, 233, 266, 280, 293, 294, 333, 408, 412, 488

* 24-hr Observation period.

Table XIII. Toxicity of VX:Calcium Hypochlorite Solutions in White Perch

Concentration	Lt50	Cumulative mortality*	Time to death
ppm	min		min
100		2/10	765, 1428
200	313	10/10	150, 271, 294, 205, 296, 297, 336, 407, 431, 499
300	212	10/10	89, 94, 134, 211, 237, 274, 288, 306, 329, 414
500	284	10/10	182, 216, 227, 292, 312, 314, 317, 320, 344, 378
1,000	222	10/10	69, 127, 172, 181, 211, 229, 307, 319, 469, 479
2,500	47	10/10	34, 35, 38, 45, 49, 50, 52, 58, 59, 62
10,000	9	10/10	6, 7, 7, 8, 8, 9, 10, 11, 12, 13

* 24-hr Observation period.

Table XIV. Toxicity of HD:Calcium Hypochlorite Solutions in White Perch

Concentration	Lt50	Cumulative mortality*	Time to death
ppm	min		min
100		0/10	
1,000		0/10	
1,500		2/10	800, 1251
1,750		0/10	
2,000	747	10/10	385, 495, 540, 645, 690, 747, 810, 1125, 1221, 1415
2,500	281	10/10	145, 192, 195, 243, 308, 339, 351, 373, 393, 429
5,000	58	10/10	31, 52, 53, 60, 62, 63, 64, 65, 66, 78
10,000	36	10/10	24, 26, 27, 29, 33, 37, 41, 41, 53, 77

* 24-hr Observation period.

Table XV. Toxicity of 10% Sodium Carbonate Solutions in Striped Bass

Concentration	Mortality*	Time to death
ppm		min
750	0/10	
875	0/10	
950	0/10	
1,000	6/10	558, 668, 743, 788, 831, 959
1,500	4/10	505, 590, 1085, 1145
2,000	10/10	202, 333, 345, 353, 358, 385, 388, 428, 505, 585
3,000	10/10	220, 265, 271, 274, 279, 315, 365, 393, 472, 485
4,000	8/10	289, 324, 350, 371, 399, 406, 409, 469

* 24-hr Observation period.

Table XVI. Toxicity of 10% Calcium Hypochlorite Solutions in Striped Bass

Concentration	Mortality*	Time to death
ppm		min
5	0/10	
8	2/10	910, 1219
9	0/20	
10	10/10	200, 345, 351, 353, 375, 385, 415, 425, 452, 471
15	0/10	
20	10/10	54, 64, 65, 77, 92, 94, 95, 97, 106, 114
80	10/10	18, 26, 30, 31, 34, 36, 36, 37, 43, 57

* 24-hr Observation period.

Table XVII. Toxicity of GB:Sodium Carbonate Solutions in Striped Bass

Concentration	Mortality*	Time to death
ppm		min
1,000	1/10	738
1,250	0/10	
1,500	6/10	390, 407, 552, 572, 812, 917
2,000	6/10	643, 670, 748, 843, 896, 1053
2,500	10/10	351, 371, 441, 466, 477, 508, 527, 538, 546, 836
4,000	10/10	99, 119, 124, 125, 126, 127, 129, 140, 164, 169
6,000	10/10	201, 220, 242, 273, 318, 328, 350, 381, 453, 513

* 24-hr Observation period.

Table XVIII. Toxicity of VX:Calcium Hypochlorite Solutions in Striped Bass

Concentration	Mortality*	Time to death
ppm		min
50	0/10	
60	0/10	
80	9/10	208, 398, 448, 498, 505, 646, 653, 940, 1068
100	10/10	671, 683, 730, 776, 848, 898, 908, 923, 988, 1028
200	0/10	
500	0/10	
1,000	0/10	
2,000	10/10	379, 424, 437, 450, 452, 453, 474, 530, 618, 704

* 24-hr Observation period.

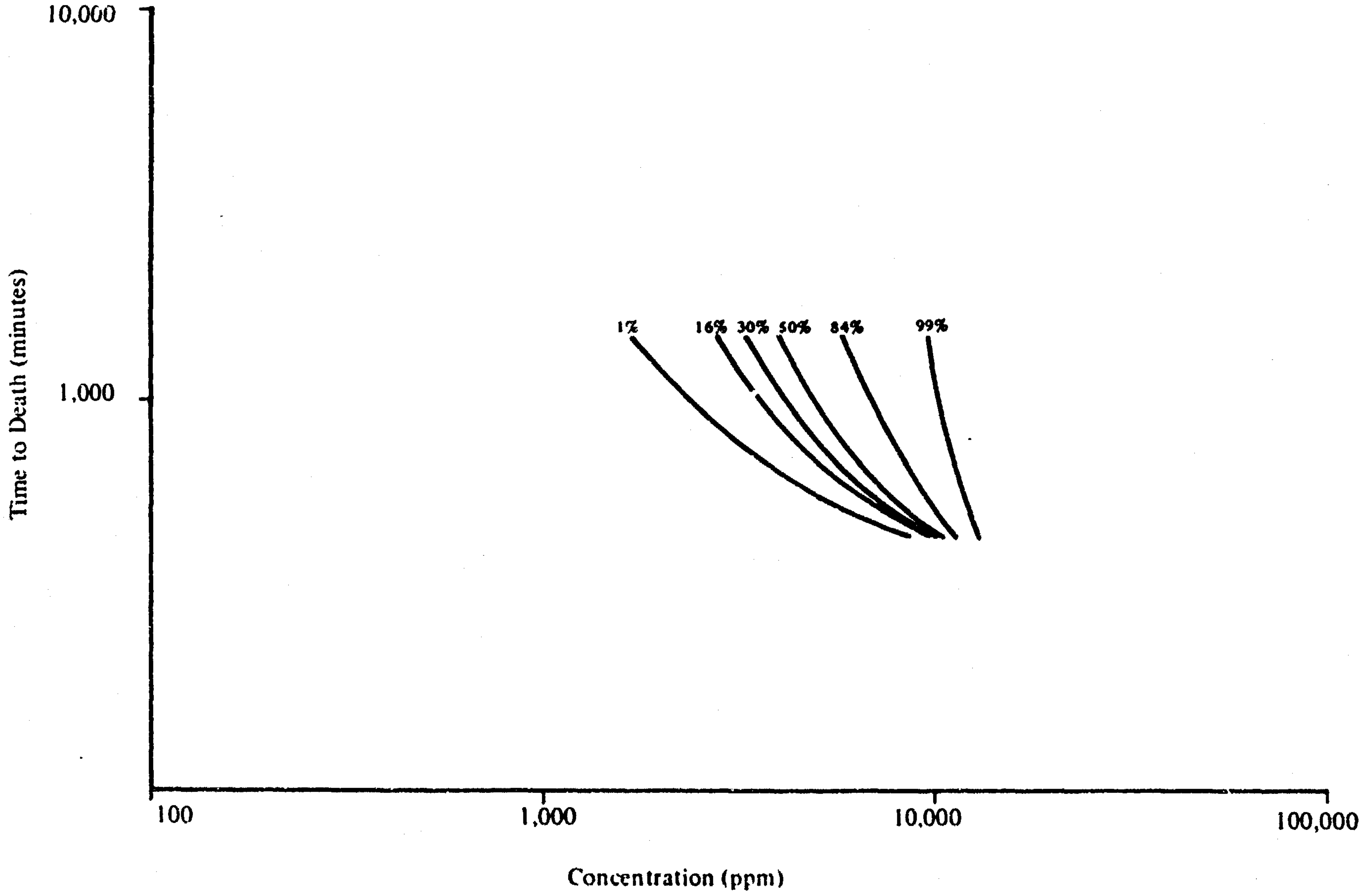

Figure 1. Toxicity of 10% Sodium Carbonate to White Perch

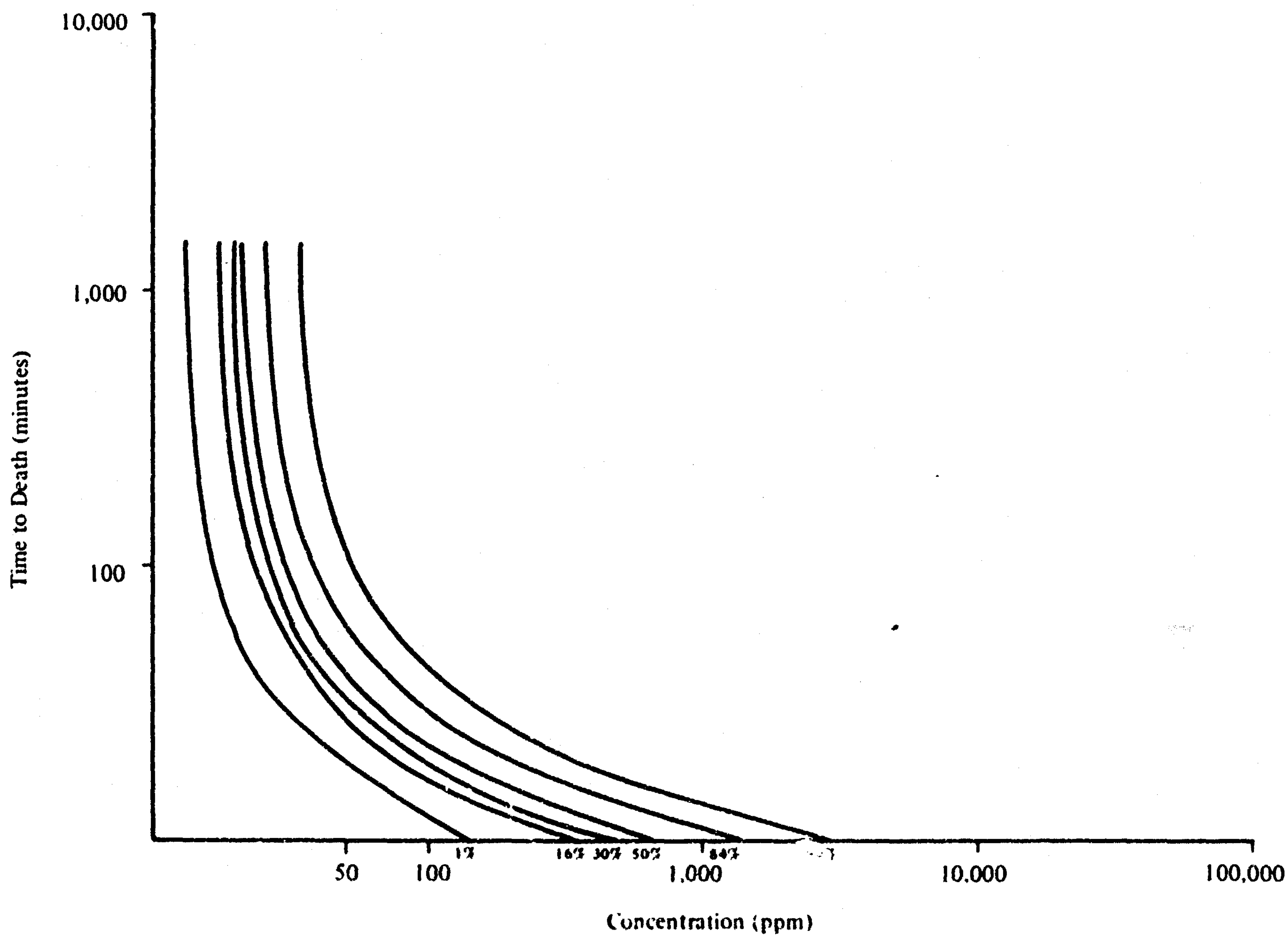

Figure 2. Toxicity of 10% Calcium Hypochlorite to White Perch

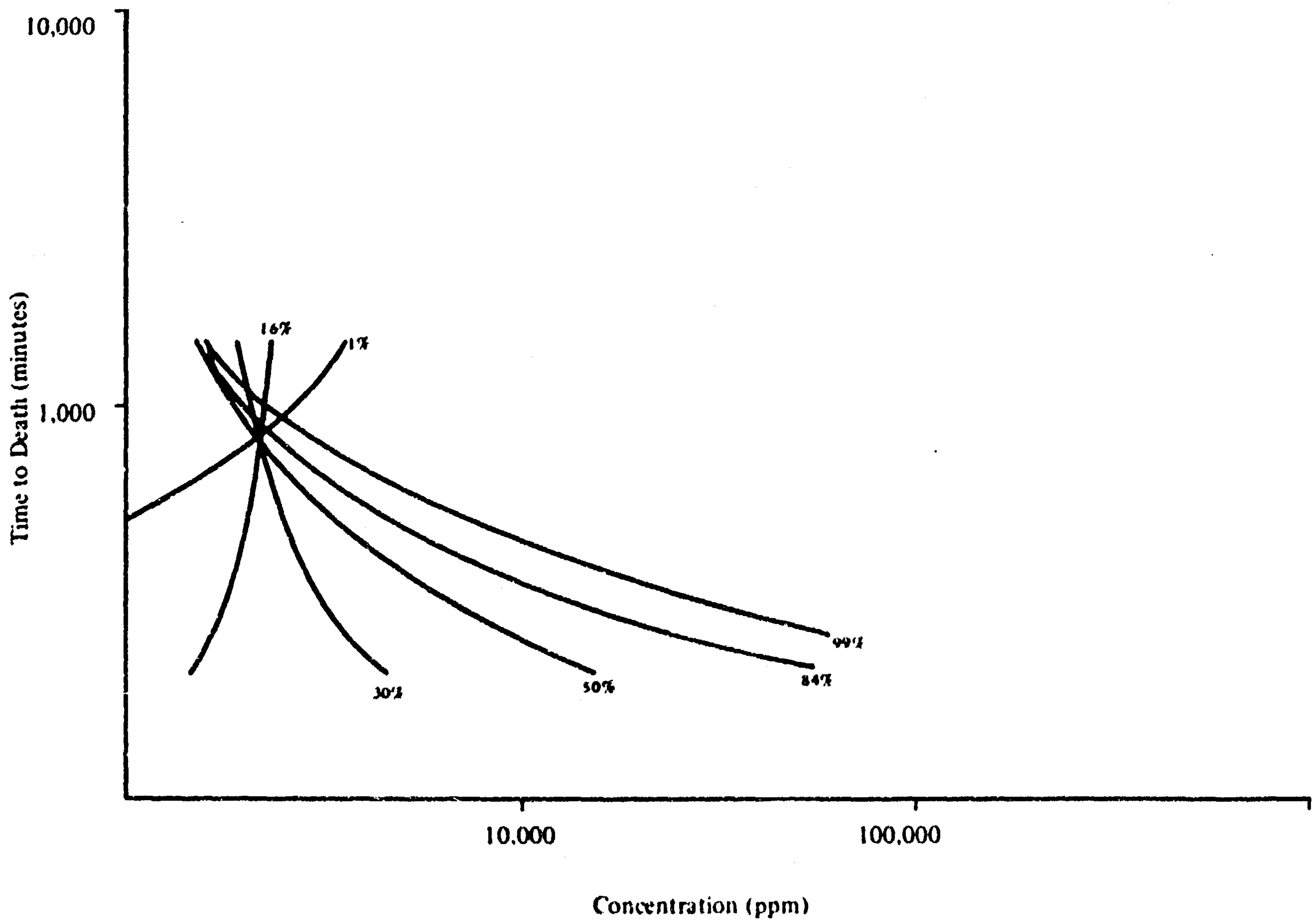

Figure 3. Toxicity of GB:Sodium Carbonate to White Perch

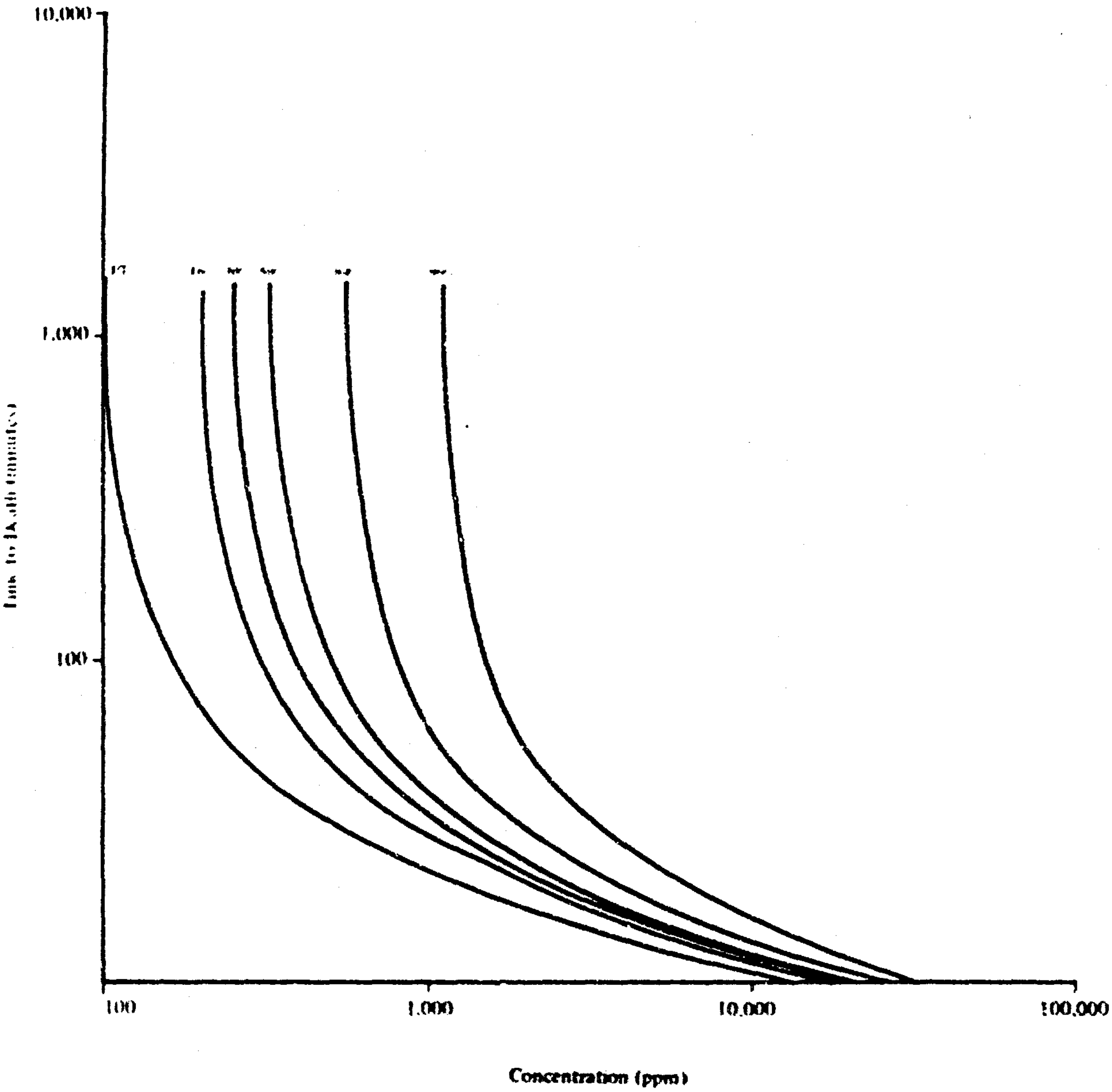

Figure 4. Toxicity of VX:Calcium Hypochlorite to White Perch

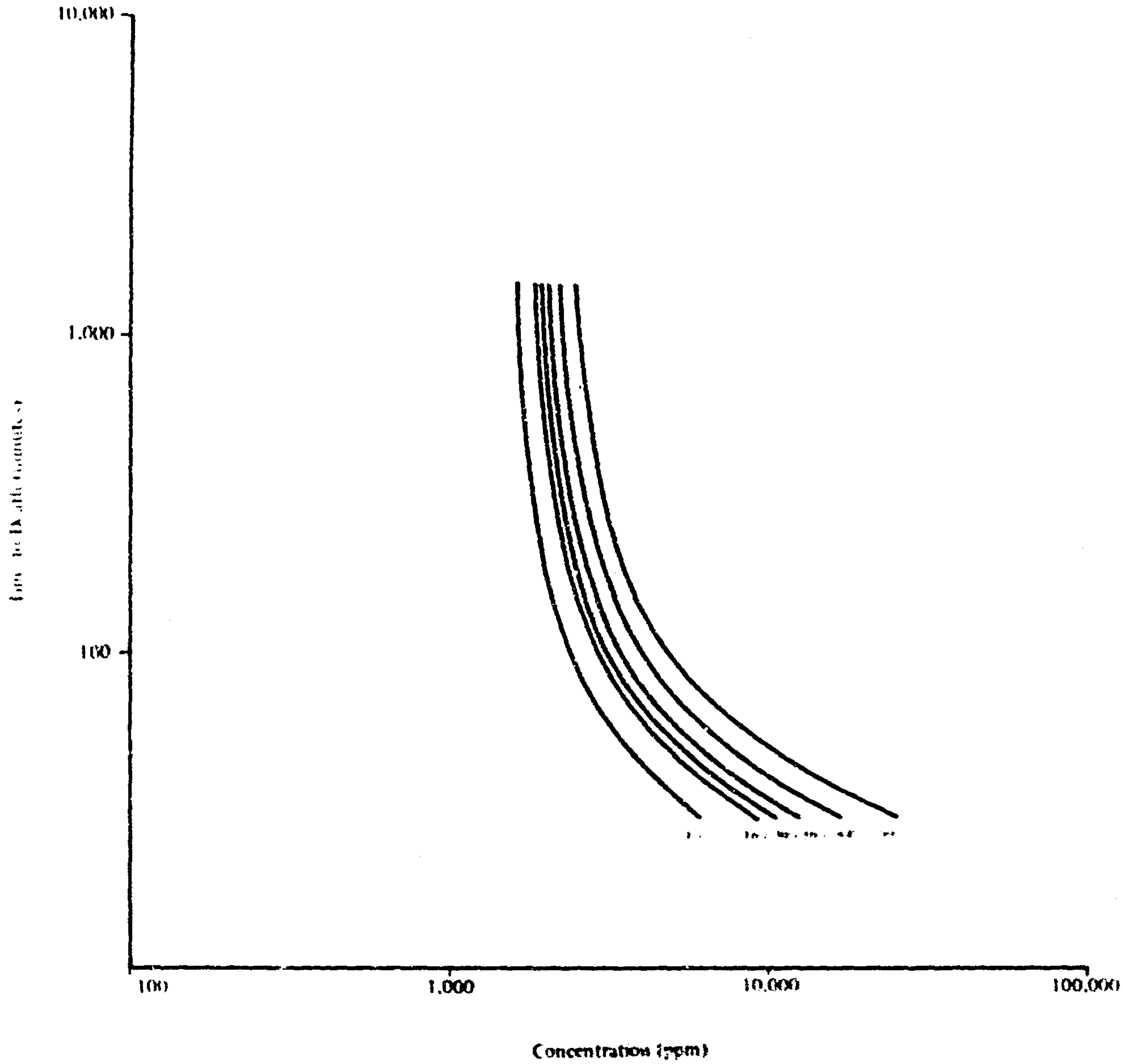

Figure 5. Toxicity of HD.Calcium Hypochlorite to White Perch

Table XIX. Toxicity of HD:Calcium Hypochlorite Solutions in Striped Bass

Concentration	Mortality*	Time to death
ppm		min
1,000	1/10	850
1,500	2/10	821, 1139
2,000	0/20	
2,500	10/10	297, 409, 428, 468, 490, 507, 527, 552, 614, 631
3,250	8/9	946, 1082, 1214, 1222, 1284, 1358, 1419, 1434
4,000	10/10	234, 237, 250, 256, 311, 328, 356, 386, 933, 1029
6,000	6/10	776, 850, 912, 1005, 1083, 1152

* 24-hr Observation period.

Table XX. Ranking of Toxicities of Unneutralized Detoxicants and Agent:Detoxicant Solutions in Mammals and Fish

Solution	Concentration required to produce 50% mortality in perch in 60 minutes	Toxicity rankings	
		Intravenous (mammals)	Total body exposure (fish)
	ppm		
10% Na_2CO_3	7,000	5	5
10% $Ca(OCl)_2$	38	1	1
GB:Na_2CO_3	2,800	4	3
VX:$Ca(OCl)_2$	600	3	2
HD:$Ca(OCl)_2$	5,000	2	4

There was no predictable dose-effect pattern in the striped bass. Despite extreme care in obtaining fresh fish of the proper weight and length and testing large numbers of controls, high concentrations of agents were sometimes less toxic than low concentrations. The relative levels of toxicity, however, were the same as for white perch and should be considered as such.

2. Aquatic Invertebrate Tests.

a. Materials and Methods.

The crustaceans assayed were the amphipod *Gammarus tigrinus*, Sexton and the glass shrimp *Palaemonetes pugio*, which are common residents of the upper Chesapeake Bay. They were collected with a beach seine and held at least a week in large aquariums before being used.

When the amphipods were tested, five to eight were transferred to 70- by 50-mm crystallizing dishes containing 100 ml of water having the same composition as that from which they were taken.* Appropriate amounts of unneutralized sodium carbonate, GB:sodium carbonate, VX:calcium hypochlorite, and HD:calcium hypochlorite were added to produce concentrations as high as 500 ppm. The dishes were covered with small watch glasses to retard evaporation, and deaths were recorded over a period of 96 hours.

When the glass shrimp were tested, six were placed in 1 liter of water in 6- by 6-inch battery jars. Other procedures were the same as for the amphipods.

Unfavorable reaction to a compound is relatively easy to detect in the microcrustacea. Increased irritability is followed by a progressive incoordination until the animal is unable to move and simply lies quivering on the bottom of the aquarium. The animal can then be considered as dead.

b. Results.

None of the amphipods died when exposed to as much as 500 ppm of the detoxified solutions or 100 ppm of sodium carbonate.

Although some of the glass shrimp exposed to the detoxified solutions died, similar mortalities were observed in control shrimp. Therefore, no toxicity could be clearly attributed to these solutions in concentrations as high as 500 ppm. Substantiating this assumption is the fact that, in some earlier studies, amphipods and glass shrimp were exposed to various concentrations of malathion, and the amphipods were found to be 50 times as sensitive as the glass shrimp to this class of compound.

D. Botanical Tests.

1. Materials and Methods.

a. Test Solutions.

Detoxified, neutralized HD, VX, and GB solutions, as described in section IIA, were used in these studies. Unneutralized sodium carbonate and calcium hypochlorite were used as negative controls. Concentrations of the diluted stock solutions are expressed as percentages of the stock concentration.

* Salinity, 2.0%; hardness, 406; pH 7.4.

Because a significant amount of residue was evident in the solutions, they were filtered before testing (the filtrate showed no significant phytotoxicity).

b. Plants.

The plants used were as follows:

Flowering plants

Wolffia papulifera Thompson, obtained from Harford County, Maryland.

Lemna perpusilla Torr., strain 6746, obtained from Dr. Jerry W. McClure, Department of Botany, Miami University, Oxford, Ohio.

Spirodela polyrhiza (L) Schleiden, obtained from Dolly Sods, West Virginia.

Floating fern

Azolla caroliniana Willd., from stock cultures already available (originally obtained commercially).

Green algae

Ourococcus bicaudatus Grobety, obtained as a contaminant of *W. papulifera* colonies.

Chlorella pyrenoidosa Chick, obtained from Dr. Richard C. Starr, Culture Collection, Indiana University, Bloomington, Indiana.

All species are indigenous to Maryland.[3]

c. Procedures.

Because preliminary testing with very dilute amounts of the detoxified unneutralized solutions usually killed the plants, presumably because of excessively high or low pH, two separate studies were done. These were to differentiate between the toxic effects of pH and the toxic effects of the agent-detoxicant solutions. In the first study, the growth medium was made acidic with hydrochloric acid or basic with potassium hydroxide, and no agent-detoxicant solutions were used. In the second study, the neutralized detoxified solutions were used. All other procedures were the same for both studies.

All plants (except *A. caroliniana*) were initially grown in 125-ml Erlenmeyer flasks, each of which contained 40 ml of Hutner's medium,* 20% of the recommended concentration. Before introduction of the plants, the flasks were closed with a cotton stopper and autoclaved for 45 minutes at 20 pounds pressure. The plants were sterilized by soaking for 5 minutes in 1% chlorine (sodium hypochlorite), after which they were transferred to the flasks.

* Hutner's medium is a combination of the basic elements needed by plants for growth. See Hillman[4] for specific ingredients. Different concentrations (5% or 20%) of stock Hutner's medium were used to fit optimum micronutrient requirements of each species.

[3] Gleason, H. A. The New Britton and Brown Illustrated Flora of the Northeastern United States and Adjacent Canada. Vol. I, pp 370-372. Lancaster Press, Lancaster, Pennsylvania. 1952.

[4] Hillman, W. S. The Induction of Flowering. L. T. Evans, ed. The Macmillan Company, Sidney, Australia. 1969.

A. caroliniana was grown in open beakers containing 5% Hutner's medium.

When the effects of pH or of the detoxified solutions were tested, the following numbers of individuals or colonies were transferred to 125-ml flasks, 10-ml beakers, or 100-ml beakers containing medium and different concentrations of the solutions (for the pH studies, the agent solutions were omitted and the acidity of the medium was adjusted with hydrochloric acid or potassium hydroxide to pH 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13):

W. papulifera: three individuals
L. perpusilla: one colony (two fronds)
S. polyrhiza: one colony (three fronds)
A. caroliniana: one individual
O. bicaudatus and *C. pyrenoidosa*: 0.2 ml of a standard colony (200 Klett units)

Table XXI shows the different photoperiods and temperatures at which the plants were grown both before subcultures were removed and when they were being tested. *W. papulifera* colonies received light continuously from both tungsten and fluorescent lights (125 ft-candles). *L. perpusilla* was initially grown under long day conditions (16 hours light/8 hours darkness) and tested with the agent:detoxicant solutions under short day conditions (10 L/14 D) so that flowering would occur. *A. caroliniana* was kept in open sunlight, the photoperiod being estimated by recording the light duration from sunrise to sunset. All other species were grown under conditions of 10 L/14 D or 14 L/10 D.

Growth of *W. papulifera* was measured by counting the number of individuals; that of the other flowering plants, by counting the number of fronds. Growth of *A. caroliniana* was determined by measuring the surface area of individual plants, and growth of the algae was determined by measuring colony density with a Klett-Summerson Photoelectric colorimeter (Model No. 1946).

Doubling time was calculated by using the method of Hillman[5] as given in an earlier paper,[6] and comparison of control versus experimental doubling time was an indication of toxicity. The approximate control doubling times (during times corresponding to the duration of the agent tests) were as follows:

W. papulifera: 2 days (pH 6.2 to 6.6)
S. polyrhiza and *L. perpusilla*: 2.5 days (pH 6.2 to 6.6)
A. caroliniana: 14 days (pH 6.2 to 6.6)
O. bicaudatus: 1 day (pH 6)
C. pyrenoidosa: 3 days (pH 6)

Because doubling times for *L. perpusilla* and *S. polyrhiza* were based on number of fronds rather than number of colonies, the figures are much lower than those reported by Bennink *et al.*[7]

5 Hillman, W. S. The Lemnaceae, or Duckweeds. Bot. Rev. 27, 221-287 (1961).

6 Worthley, E. G., and Schott, C.D. EATR 4595. The Comparative Effects of CS and Various Pollutants on Fresh Water Phytoplankton Colonies of *Wolffia papulifera* Thompson. December 1971. UNCLASSIFIED Report.

7 Bennink, G. J. H., Van Den Berg, R., Cool, H. J., and Stegwee, D. Flowering in *Lemna minor*. Acta Bot. Neerl. 19, 384-392 (1970).

Table XXI. Initial and Experimental Growth Conditions for Plants

Species	Photoperiod[a]		Container		Temperature[b]	
	Initial[c]	Experimental	Initial[c]	Experimental	Initial[c]	Experimental
					°C	
W. papulifera	Continuous	Continuous	Sealed[d]	Open[e]	22-30	22-30
L. perpusilla	16L/8D	10L/14D	Sealed	Sealed	27	27
S. polyrhiza	10L/14D	10L/14D	Sealed	Sealed	27	27
A. caroliniana	14L/10D	14L/10D	Open	Open	22-30	22-30
O. bicaudatus	14L/10D	10L/14C[f]	Sealed	Open	22-30	27
C. pyrenoidosa	14L/10D	10L/14D	Sealed	Open	22-30	27

[a] L = hours of darkness, D = hours of light.
[b] When the temperature was 27°C, plants were in a Sherer Model CEL 25-7 HL controlled environment chamber.
[c] Before subcultures were tested.
[d] In sterile conditions, in 125-ml flasks.
[e] In open 10- or 100-ml beakers.
[f] C = control.

2. Results.

In the tests of the effects of acidity (no agents), most of the plants tolerated pH 4, but none tolerated more acid media. Most tolerated pH 5 through 10, but not pH 11, 12, and 13. These data are plotted in figures 6 through 11.

When the plants were grown in media containing the detoxified, neutralized solutions and the unneutralized detoxicants, the latter were the most toxic of the group, based on the concentration that had no effect (table XXII).

All three agent-detoxicant solutions killed *W. papulifera*, *L. perpusilla*, and *A. caroliniana* at 1% of the initial stock concentration and *C. pyrenoidosa* at 0.1% of the stock concentration. *O. bicaudatus* was killed at 0.1% of the stock concentrations of HD and GB solutions, but the VX solution was lethal only at the 1% stock concentration. These data are shown in table XXIII.

The growth rates of some plants were actually increased at these concentrations:

VX, 0.01%: *W. papulifera*, *A. caroliniana*, and *L. perpusilla*

HD, 0.001%: *L. perpusilla* and *A. caroliniana*

GB, 0.01%: *L. perpusilla*

These data show that all the detoxified solutions tested should be neutralized before entering water systems. Dilution of the GB and VX solutions 100,000-fold and dilution of the HD solution 1,000,000-fold would neutralize them.

The low pH of detoxified HD is especially detrimental to plant life. But disposal of basic solutions also poses a potentially serious problem. Because the growth rates of the algae *O. bicaudatus* and *C. pyrenoidosa* were increased at basic pH's, unneutralized GB solution could stimulate the growth of basophilic algae and cause a "water bloom." Excessive algal growth clogs the water, kills fish and other aquatic organisms, causes the water to have a bad odor, depresses the oxygen levels, and upsets the ecological balance of a lake, river, or ocean.[8-11]

Dilution of these detoxified solutions and disposal in salt water[12,13] would be least harmful to the environment.

[8] Carr, D. E. Death of Sweet Waters. W.W. Norton Company, Inc., New York, New York. 1966.

[9] Simpson, G. G., Pittendrigh, C. S., and Tiffany, L. H. Life: An Introduction to Biology. Harcourt, Brace and World, Inc., New York, New York.

[10] Wilber, C. G. The Biological Aspects of Water Pollution. C. C. Thomas, Springfield, Illinois. 1970.

[11] Demek, M. M., Davis, G. T., Dennis, W. H., Jr., Hill, A. L., Farrand, R. L., Musselman, N. P., Mazza, R. J., Devine, W. D., Rosenblatt, D. H., and Epstein, J. EATR 4417. Behavior of Chemical Agents in Seawater. August 1970. UNCLASSIFIED Report.

[12] Epstein, J. Rate of Decomposition of GB in Seawater. Science 170, 1396 (1970).

[13] Gleason, M. M., Gosselin, R. E., and Hodge, M. C. Clinical Toxicology of Commercial Products. Williams and Wilkins Company, Baltimore, Maryland. 1957.

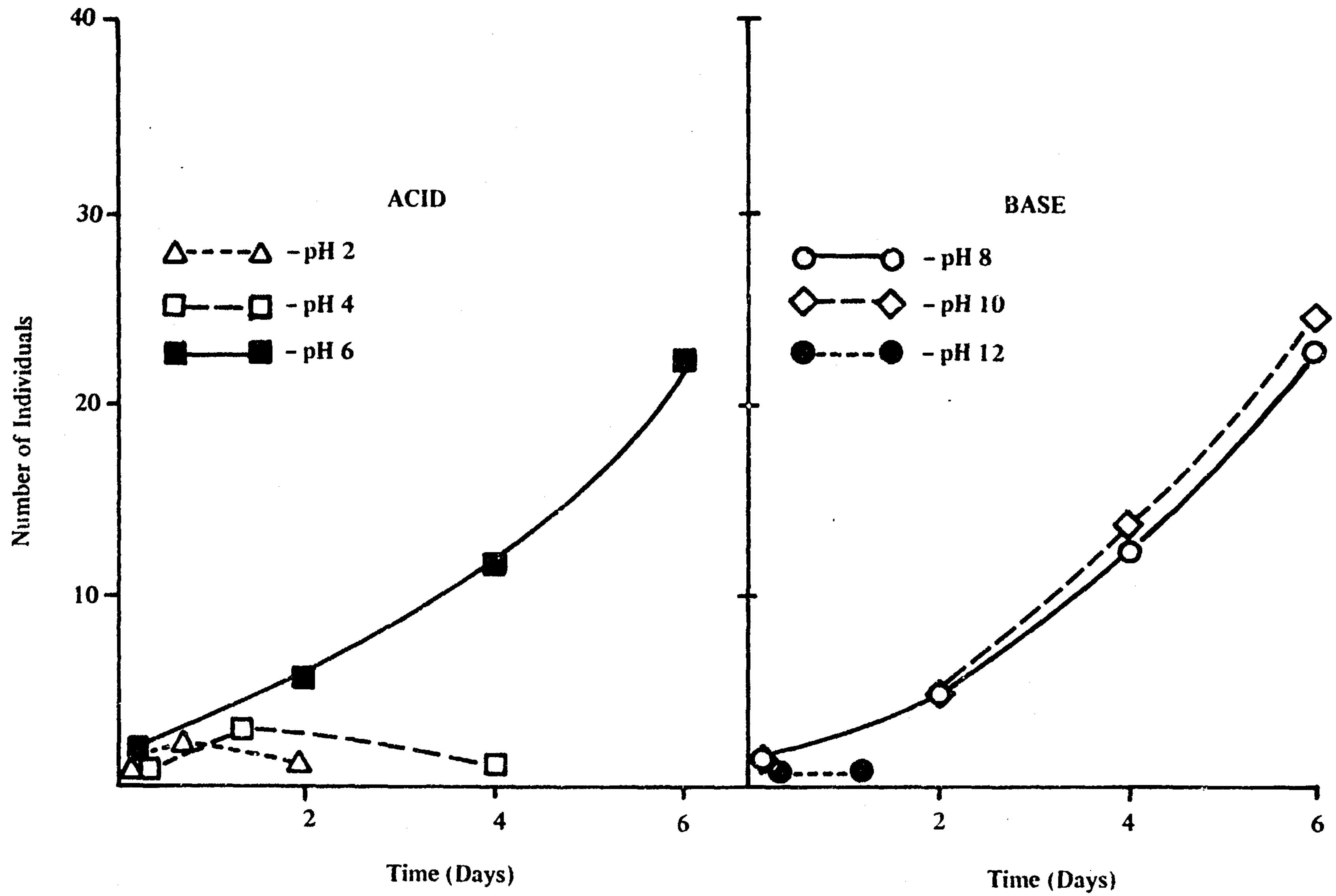

Figure 6. Response of *Wolffia papulifera* to Varying Initial pH of Hutner's Medium at 27°C

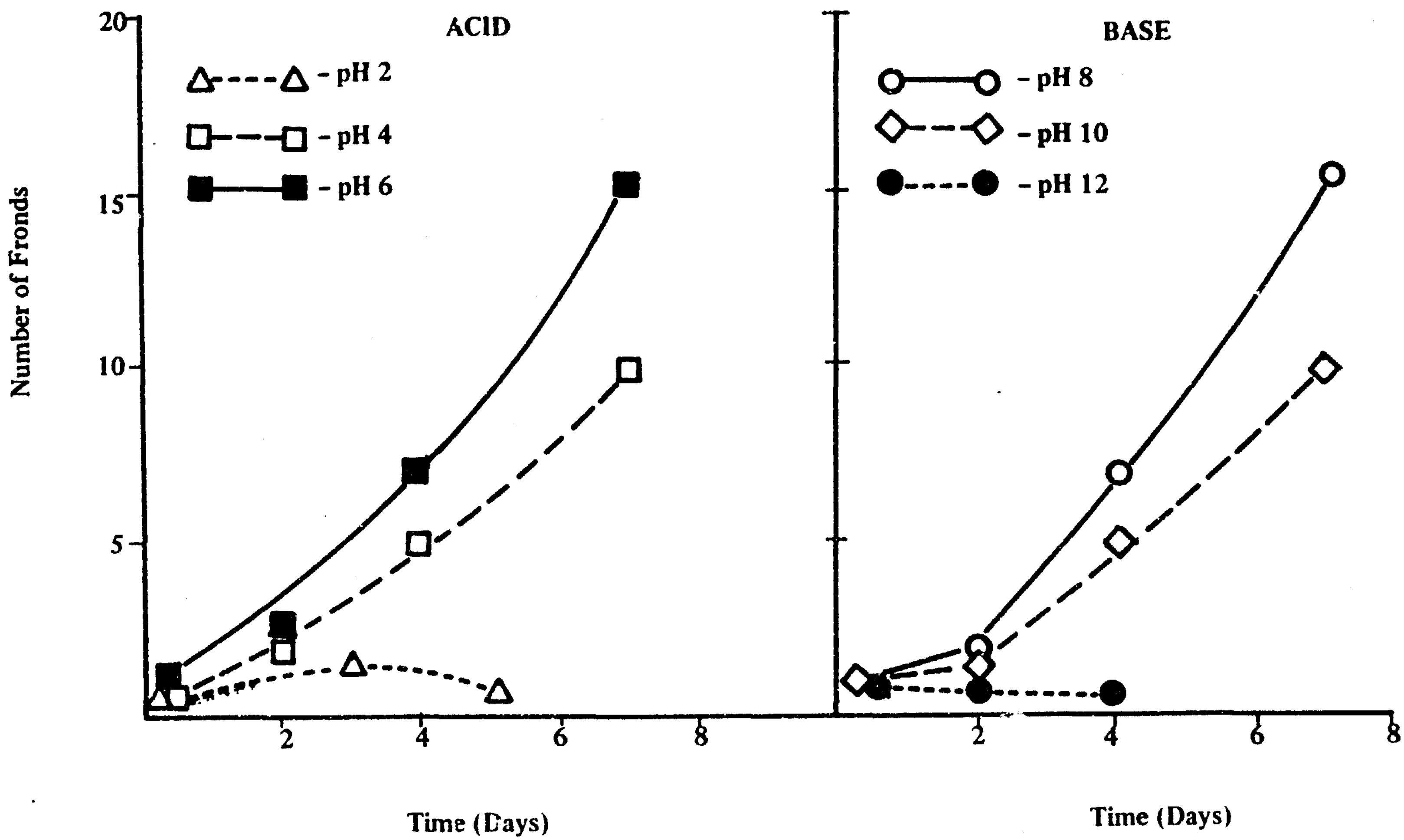

Figure 7. Response of *Lemna perpusilla* to Varying Initial pH of Hutner's Medium at 27°C

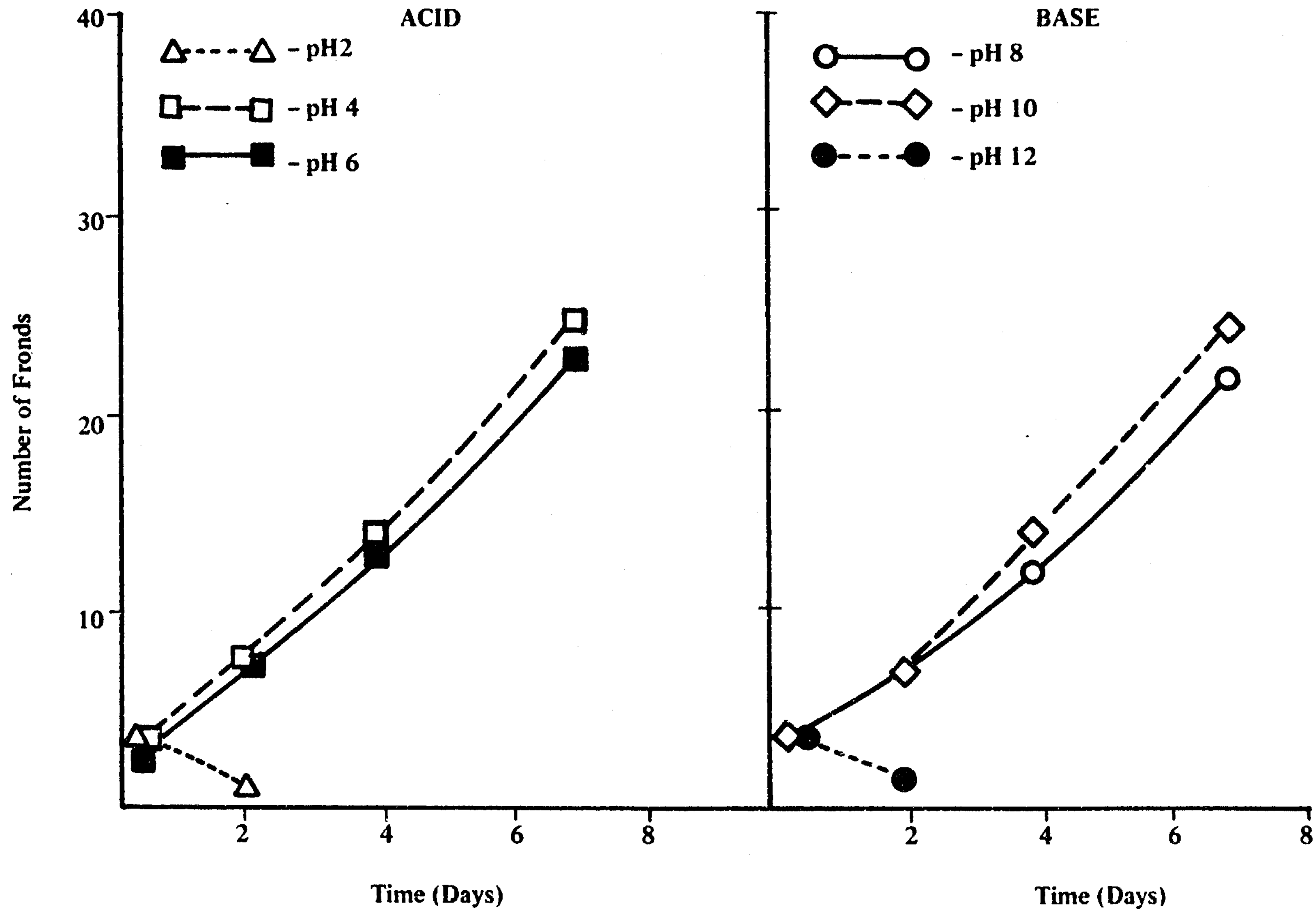

Figure 8. Response of *Spirodela polyrhiza* to Varying Initial pH of Hutner's Medium at 27°C

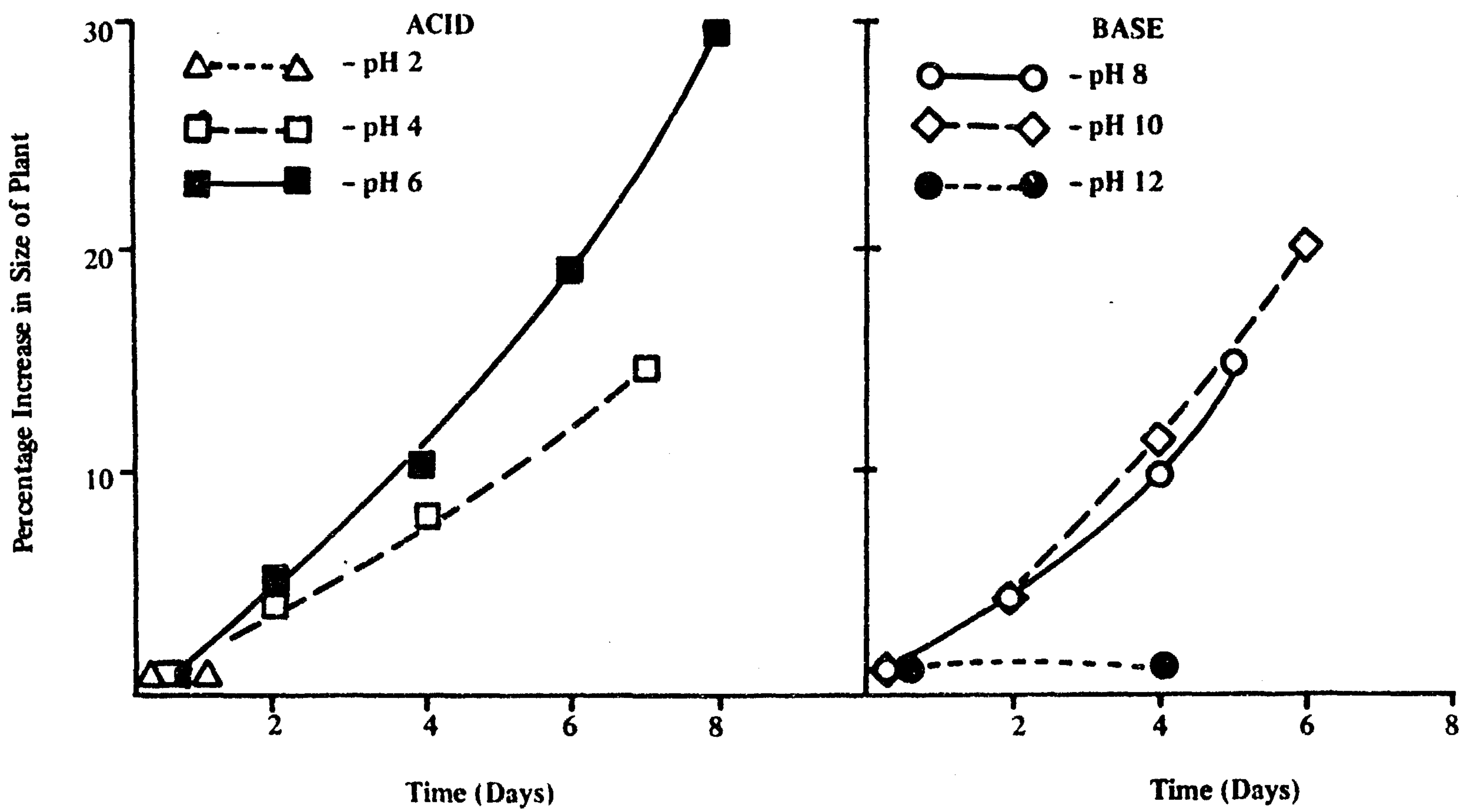

Figure 9. Response of *Azolla caroliniana* to Varying Initial pH of Hutner's Medium at 27°C

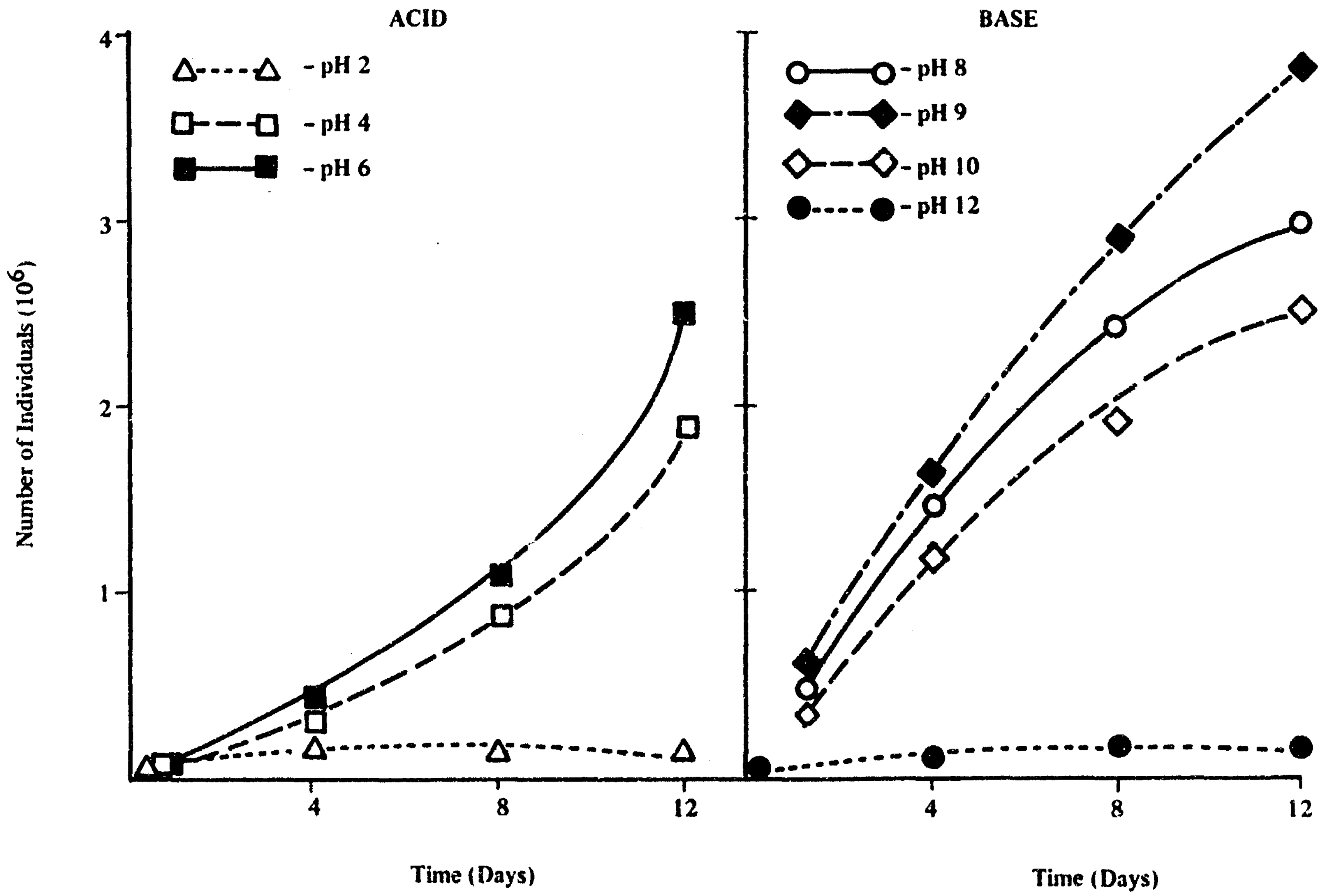

Figure 10. Response of *Ourococcus bicaudatus* to Varying Initial pH of Hutner's Medium at 27°C

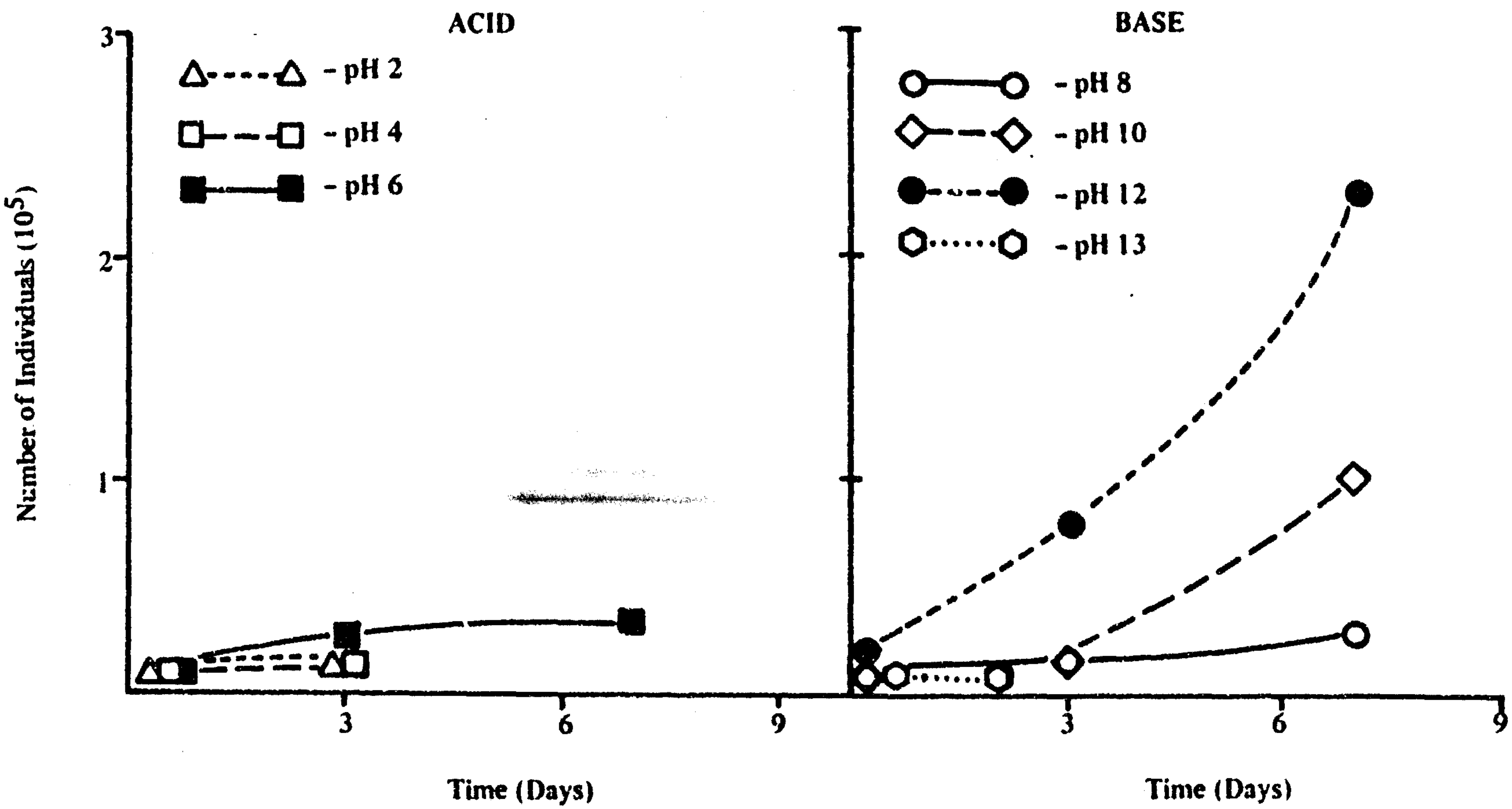

Figure 11. Response of *Chlorella pyrenoidosa* to Varying Initial pH of Hutner's Medium at 27°C

Table XXII. No-Effect Concentrations of Detoxicants and Detoxified Agent Solutions on Plants

Plant	No-effect concentration				
	$VX:Ca(OCl)_2$	$HD:Ca(OCl)_2$	$Ca(OCl)_2$	$GB:Na_2CO_3$	Na_2CO_3
	% of initial solution				
W. papulifera	0.1, 0.001	0.01	0.0001	0.1	0.001
L. perpusilla	0.1, 0.001	0.0001	0.00001	0.001	0 001
S. polyrhiza	–	0.001	0.00001	–	–
A. caroliniana	0.001	0.0001	0.0001	0.001	0.001
O. bicaudatus	0.01	0.01	0.0001	0.01	0.001
C. pyrenoidosa	0.01	0.01	0.0001	0.01	0.001

Table XXIII. Plant Responses at Each Concentration of Detoxified Neutralized VX, HD, and GB Tested

Chemical	Concentration tested	Effect	Test duration
	%		days
VX:Ca(OCl)$_2$			
		W. papulifera	
	1.0	Death	3
	[illegible]	No effect	11
	0.01	Increased growth rate	11
	0.001	No effect	11
		L. perpusilla	
	1.0	Death	3
	0.1	No effect	8
	0.01	Increased growth rate and flowering percent	8
	0.001	No effect	8
		A. caroliniana	
	1.0	Death	3
	0.1	Decreased growth rate	5
	0.01	Increased growth rate	5
	0.001	No effect	5
		O. bicaudatus	
	1.0	Death	1
	0.1	Decreased growth rate	7
	0.01	No effect	7
	0.001	No effect	7
		C. pyrenoidosa	
	1.0	Death	1
	0.1	Death	1
	0.01	No effect	7
	0.001	No effect	7
HD:Ca(OCl)$_2$			
		W. papulifera	
	1.0	Death	3
	0.1	Decreased growth rate	11
	0.01	No effect	11
	0.001	No effect	11

Table XXIII (Contd)

Chemical	Concentration tested	Effect	Test duration
	%		days
		L. perpusilla	
	1.0	Death	3
	0.1	Decreased growth rate	8
	0.01	Decreased growth rate	8
	0.001	Increased growth rate and flowering percent	8
	0.0001	No effect	8
		S. polyrhiza	
	1.0	Death	4
	0.1	Decreased growth rate	4
	0.01	Decreased growth rate	4
	0.001	No effect	4
		A. caroliniana	
	1.0	Death	3
	0.1	Decreased growth rate	5
	0.01	Decreased growth rate	5
	0.001	Increased growth rate	6
	0.0001	No effect	5
		O. bicaudatus	
	1.0	Death	1
	0.1	Death	1
	0.01	No effect	7
	0.001	No effect	7
		C. pyrenoidosa	
	1.0	Death	1
	0.1	Death	1
	0.01	No effect	7
	0.001	No effect	7
GB:Na_2CO_3			
		W. papulifera	
	1.0	Death	3
	0.1	No effect	11
	0.01	No effect	11
	0.001	No effect	11
		L. perpusilla	
	1.0	Death	3
	0.1	Decreased growth rate	8
	0.01	Increased growth rate and flowering percent	8
	0.001	No effect	8
		A. caroliniana	
	1.0	Death	3
	0.1	Decreased growth rate	5
	0.01	Decreased growth rate	5
	0.001	No effect	5

Table XXIII (Contd)

Chemical	Concentration tested	Effect	Test duration
	%		days
		O. bicaudatus	
	1.0	Death	[illegible]
	0.1	Death	1
	0.01	No effect	7
	0.001	No effect	7
		C. pyrenoidosa	
	1.0	Death	1
	0.1	Death	1
	0.01	No effect	7
	0.001	No effect	7

III. DISCUSSION.

Intravenous and intragastric tests in animals showed unneutralized 10% calcium hypochlorite to be the most toxic of the 10 solutions tested. The intravenous LD50's of the solutions ranged from 0.18 to >31.6 ml/kg in the four animal species tested. The intragastric LD50's ranged from 13.3 to 56.9 ml/kg for the three species tested. Neutralization had little effect on reducing potency; in fact, in some cases it increased potency. The detoxified solutions, however, were only 1×10^{-2} to 1×10^{-5} times as potent as undetoxified agents.

All of the solutions must be considered to be extremely toxic to man according to the rating scale proposed by Gleason, Gosselin, and Hodge.[13] To reduce their toxicities to "slightly" or "practically nontoxic," the solutions must be diluted 1:1,000 to 1:10,000. Dilution should be required when the materials are to be handled by unprotected people or if the solutions are to be released into fresh water supplies.

Only the unneutralized 10% calcium hypochlorite and 10% sodium carbonate proved to be irritating to the rabbit eye. The calcium hypochlorite had the most drastic effect, including scarring of the cornea, but this damage resolved within 3 weeks. In the skin studies, only the unneutralized calcium hypochlorite produced irritation. Erythema appeared within 24 hours and necrosis within 48 hours. Patches of eschar could be seen for 14 days, but after 20 days all skin areas were normal. None of the 10 solutions produced any signs of systemic toxicity when applied to the eyes and skin. However, the same body protection prescribed for handling corrosive liquids should be worn by people handling the undiluted materials.

Studies with fish showed that disposal of the unneutralized solutions into inhabited waters would cause deaths. Dilution of unneutralized 10% calcium hypochlorite to <10 ppm and dilution of the other nine solutions to <50 ppm would preclude fish kills

Toxicity tests on five aquatic plants show that even more stringent precautions must be taken if these plants are to be spared. Since all but one of the test plants died when cultured in medium at pH 3, the disposal of unneutralized, detoxified HD would probably kill most of the aquatic plant life present. Because a basic pH stimulated the growth of algae, disposal of unneutralized detoxified VX and GB would probably cause a "water bloom," clogging water, killing fish and other aquatic organisms, causing the water to have a bad odor, and depressing the oxygen level of the water.

Based on the studies with the aquatic plants, all these solutions should be neutralized and diluted before disposal. Dilution of the GB and VX solutions 100,000-fold and dilution of the HD solution 1,000,000-fold would make their disposal safe.

IV. CONCLUSIONS.

VX, GB, and HD detoxified by presently proposed procedures must be handled as follows:

1. Personnel handling the unneutralized solutions should wear the same body protection prescribed for handling corrosive liquids.

2. The solutions should be diluted 1:1,000 to 1:10,000 before being handled by unprotected personnel.

3. Unneutralized 10% calcium hypochlorite should be diluted to <10 ppm and the other nine solutions should be diluted to <50 ppm before release into waters inhabited by fish.

4. All solutions must be neutralized before being released into waters containing aquatic plants; the GB and VX solutions should also be diluted 1:100,000 and the HD should be diluted 1:1,000,000.

V. LIMITATIONS ON USE OF THESE DATA.

These tests were done for one purpose: to develop toxicity data for use in establishing procedures for intermittent disposal of small lots of detoxified VX, GB, and HD in brackish or salt water streams. The plants and aquatic species tested are indigenous to North America. Also, because it was anticipated that enough time would elapse between "dumps" to avoid buildups in the water, only acute studies were done. Thus, the effects of chronic exposure to the levels proposed are not known.

These facts should be taken into consideration in any attempt to apply the data to areas other than brackish or salt water streams in North America or to a long-term disposal of large amounts of the solutions studied.

LITERATURE CITED

1. Illustrated Guide for Grading Eye Irritation by Hazardous Substances. Food and Drug Administration. November 1967.

2. Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics. Association of Food and Drug Officials of the United States, Baltimore, Maryland. 1959.

3. Gleason, H. A. The New Britton and Brown Illustrated Flora of the Northeastern United States and Adjacent Canada. Vol. I. pp 370-372. Lancaster Press, Lancaster, Pennsylvania. 1952.

4. Hillman, W. S. The Induction of Flowering. L. T. Evans, ed. The Macmillan Company, Sidney, Australia. 1969.

5. Hillman, W. S. The Lemnaceae, or Duckweeds. Bot. Rev. 27, 221-287 (1961).

6. Worthley, E. G., and Schott, C. D. EATR 4595. The Comparative Effects of CS and Various Pollutants on Fresh Water Phytoplankton Colonies of *Wolffia papulifera* Thompson. December 1971. UNCLASSIFIED Report.

7. Bennink, G. J. H., Van Den Berg, R., Cool, H. J, and Stegwee, D. Flowering in *Lemna minor*. Acta Bot. Neerl. 19, 384-392 (1970).

8. Carr, D. E. Death of Sweet Waters. W. W. Norton Company, Inc., New York, New York. 1966.

9. Simpson, G. G., Pittendrigh, C. S., and Tiffany, L. H. Life: An Introduction to Biology. Harcourt, Brace and World, Inc., New York, New York.

10. Wilber, C. G. The Biological Aspects of Water Pollution. C. C. Thomas, Springfield, Illinois. 1970.

11. Demek, M. M., Davis, G. T., Dennis, W. H., Jr., Hill, A. L., Farrand, R. L., Musselman, N. P., Mazza, R. J., Devine, W. D., Rosenblatt, D. H., and Epstein, J. EATR 4417. Behavior of Chemical Agents in Seawater. August 1970. UNCLASSIFIED Report.

12. Epstein, J. Rate of Decomposition of GB in Seawater. Science 170, 1396 (1970).

13. Gleason, M. M., Gosselin, R. E., and Hodge, M. C. Clinical Toxicology of Commercial Products. Williams and Wilkins Company, Baltimore, Maryland. 1957.

APPENDIX

TABLES

Table A-1. Toxicity of Sodium Carbonate and GB:Sodium Carbonate in the Mouse, Rat, Rabbit, and Frog

Species and route	Na_2CO_3 LD50								$GB:Na_2CO_3$ LD50									
	As received (pH 12.0)				Neutralized (pH 7.0)				As received (pH 9.8)					Neutralized (pH 7.0)				
	No. of animals	Slope	ml/kg	mg/kg a/	No. of animals	Slope	ml/kg	mg/kg a/	No. of animals	Slope	ml/kg	mg/kg b/	mg/kg c/	No. of animals	Slope	ml/kg	mg/kg b/	mg/kg c/
Mouse																		
IV	50	10.6762	4.70	470	50	15.2149	13.8	1380	50	11.0951	7.13	117	830	60	11.0066	7.78	128	906
IG	60	17.7803	43.7	4370	60	24.1584	38.0	3800	50	28.1852	36.9	605	4295	50	16.3176	27.7	454	3224
Rat																		
IV	30	11.2898	3.00	300	30	11.4489	3.53	353	30	27.4734	2.63	43	306	36	8.5047	2.78	46	324
IG	24	50.2291	31.9	3190	24	52.3493	29.3	2930	30	16.9719	24.3	399	2829	30	32.5153	27.8	456	3236
Rabbit																		
IV	16	d/	1.78	178	16	d/	8.14	814	16	d/	1.78	29.2	207	16	d/	5.62	92.2	654
IG	16	d/	21.5	2150	16	d/	14.6	1460	16	2.4371	30.4	499	3539	16	d/	13.3	218	1548
Frog																		
Dorsal lymph sac	16	c/	>31.6	>3160	16	d/	>28.6	>2860	16	d/	31.6	518	3678	16	d/	>31.6	>518	>3678

a/ Milligrams of Na_2CO_3 per kilogram.

b/ Milligrams of agent originally in the volume of the LD50.

c/ Calculated as mg/kg of total solids, weight of agent + decontaminant.

d/ Assay done according to RL SOP 70-3 using minimum number of animals; no slope can be drawn.

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Table A-II. Toxicity of Calcium Hypochlorite, VX:Calcium Hypochlorite, and HD:Calcium Hypochlorite in the Mouse, Rat, Rabbit, and Frog

Species and route	$Ca(OCl)_2$ LD50 As received (pH 12.0) No. of animals	Slope	ml/kg	mg/kg[a]	Neutralized (pH 7.0) No. of animals	Slope	ml/kg	mg/kg[a]
Mouse								
IV	70	11.4685	0.86	86	70	24.1041	2.38	238
IG	40	34.1393	21.0	2100	50	39.7793	40.6	4060
Rat								
IV	24	11.9157	0.18	18	24	12.6585	1.03	103
IG	24	6.4188	13.9	1390	24	20.8849	30.3	3030
Rabbit								
IV	16	d/	0.18	18	16	d/	0.51	51
IG	16	d/	17.8	1780	16	d/	21.5	2150
Frog								
Dorsal lymph sac	16	d/	1.78	178	16	d/	>30.0	>3000

Species and route	$VX/Ca(OCl)_2$ LD50 As received (pH 6.3) No. of animals	Slope	ml/kg	mg/kg[b]	mg/kg[c]	Neutralized (pH 7.0) No. of animals	Slope	ml/kg	mg/kg[b]	mg/kg[c]
Mouse										
IV	60	12.9768	2.39	39	278	80	8.8283	2.36	39	275
IG	60	20.7530	48.2	790	5610	50	14.5030	56.9	933	6623
Rat										
IV	24	13.3831	1.22	20	142	24	21.8299	1.39	23	162
IG	24	42.3164	40.8	669	4749	24	58.7451	41.2	676	4796
Rabbit										
IV	16	2.6876	0.74	12	86	16	d/	0.56	9.18	65
IG	16	d/	23.7	389	2759	16	d/	17.8	292	2072
Frog										
Dorsal lymph sac	16	d/	>31.6	>518	>3678	16	d/	>31.6	>518	>3678

Species and route	$HD/Ca(OCl)_2$ LD50 As received (pH 1.9) No. of animals	Slope	ml/kg	mg/kg[b]	mg/kg[c]	Neutralized (pH 7.0) No. of animals	Slope	ml/kg	mg/kg[b]	mg/kg[c]
Mouse										
IV	60	18.6791	1.99	24	223	70	18.2276	2.17	26.0	243
IG	60	32.3809	39.8	478	4458	50	35.6356	36.0	432	4032
Rat										
IV	24	22.4030	0.70	8	78	30	11.9461	0.75	9	84
IG	24	39.1224	29.7	356	3326	36	15.3643	31.7	380	3550
Rabbit										
IV	16	d/	1.78	21.4	199	16	d/	1.33	16	149
IG	16	d/	31.6	379	3539	16	d/	17.8	214	1994
Frog										
Dorsal lymph sac	16	d/	31.6	379	3539	16	d/	>31.6	>379	>3539

a/ Milligrams of $Ca(OCl)_2$ per kilogram.

b/ Milligrams of agent originally in the volume of the LD50.

c/ Calculated as mg/kg of total solids, weight of agent + decontaminant.

d/ Assay [illegible] e according to RL SOP 70-3 using minimum number of animals; no slope can be drawn.